Title: Maternal rates of lipolysis and glucose production in late pregnancy are independently related to fetal weight

Short title: Late Pregnancy Glucose Production and Lipolysis

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Keywords: lipolysis; glucose production; maternal glycaemia; fetal weight; resting energy expenditure

Acknowledgements: We thank all the pregnant women who participated in the study. We also thank the staff at the Addenbrooke's Wellcome Trust Clinical Research Facility, and sonographers (Trish Chudleigh) as well as midwives and staff at the diabetic clinic (lead nurse Kerry Stubbington and Dr David Simmons), Rosie Maternity Hospital, Cambridge, the National Institute for Health Research (NIHR) Cambridge Comprehensive Biomedical Research Centre and NHS Research and Development**.** We are grateful for technical assistance from Angie Watts, Cambridge and Elisabet Söderberg, Uppsala. Les Bluck, Antony Wright and Marilena Leventi (MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge), for analysis of total body water measurements. We thank Dr Ken Ong (MRC Epidemiology Unit, Cambridge) and the late Dr Björn Jonsson (Department of Women's and Children's Health, Uppsala University) for support in the statistical analysis.

Grants: This project was supported by research grants from Diabetes UK and Funds and Friends of Addenbrooke's. Dr Diderholm was supported by the Throne-Holst Foundation, Uppsala University, Her Royal Highness the Crown Princess Lovisa's Foundation for Children's Health Care, the Wera Ekström Foundation, the Gillberg Foundation, the Swedish Society of Medical Research and the Swedish Society of Medicine.

Conflicting interests: None of the authors have a conflict of interest

Financial disclosure: Nothing to declare

Word count: abstract 234 (250) words, manuscript 3090 (3500) words, references 34 (35), tables 3 (6)

Abstract

Objective: Associations between maternal glucose levels and increased fetal growth are well established and independent relationships with maternal weight, weight gain and insulin resistance are also observed. The relative roles of lipolysis and glucose production in the determination of these observations remains unclear.

Design: We examined, through detailed physiological studies, the relationship between maternal late gestational energy substrate production (glucose and glycerol) maternal weight and weight gain with estimated fetal size in the third trimester.

Patients: Twenty-one nulliparous pregnant women, without gestational diabetes (GDM) assessed at 28 weeks with oral glucose tolerance test, were recruited.

Measurements: Rates of hepatic glucose production (GPR) and rates of glycerol production (reflecting lipolysis) using [$^{13}C_6$]-glucose and [$^{2}H_5$]-glycerol were measured at 34-36 weeks gestation. Respiratory quotient was assessed by indirect calorimetry and body composition by measurements of total body water ($H_2{}^{18}O$) and body density (BODPOD). Fetal weight was estimated from ultrasound measures of biparietal diameter, femoral length and abdominal circumference.

Results: At 34-36 weeks bivariate analyses showed that GPR and lipolysis correlated with estimated fetal (r=0.71 and 0.72 respectively) as well as with maternal weight, fat mass and fat free mass, but not maternal weight gain. In multivariate analyses both rates of glucose production (r=0.43*) and lipolysis (r=0.46*) were independently associated with fetal size explaining 60% of the variance.

Conclusions: Both maternal rates of lipolysis and hepatic glucose production in late gestation are strongly related to estimated fetal weight.

Introduction

The metabolic environment during intrauterine life is known to influence health of the offspring. Impaired glucose tolerance in pregnancy, resulting from gestational, type 1 and type 2 diabetes, is associated with risk of macrosomia and may have important implications for risk of obesity and type 2 diabetes in the offspring. Data from the Hyperglycaemia and Adverse Pregnancy Outcome study (HAPO) has clearly shown a continuous linear relationship between maternal glucose levels and the weight of the newborn infant.[1] Independent effects of maternal body mass index (BMI),[2] insulin resistance[3] and basal metabolic rate[4] have also been suggested as important factors in determining fetal growth.

Since fetal overgrowth is common also in diabetic pregnancies with adequate glucose control, placental transfer of substrates other than glucose could be involved in determining weight and body composition of the fetus.[2, 5, 6]

During pregnancy levels of non-esterified fatty acids (NEFA), triacylglycerols and cholesterol increase in the circulation. These correlate positively with maternal BMI and insulin resistance as well as fetal macrosomia even in the absence of diagnosed gestational diabetes.[2, 7-9] There is only limited information on the relative roles of lipolysis and glucose production on fetal size. Thus, we performed a detailed physiological study in a cohort of healthy non-diabetic women with varying levels of glucose tolerance, focusing on the relative importance of maternal rates of lipolysis and glucose production for fetal growth.

Methods

Subjects

Subjects were recruited from the Cambridge Baby Growth Study, a study of maternal and early childhood growth and development.[10] The women were approached at 28 weeks of

gestation during a routine oral glucose tolerance test (OGTT), which is part of the Cambridge Baby Growth Study protocol, and from the gestational diabetes outpatient clinic at Rosie Maternity Hospital, Cambridge. Written consent was obtained after oral and written information had been provided. The study was approved by the Local Research Ethics Committee UK.

Dating of the pregnancies was performed by measurement of the crown rump length or biparietal diameter at routine ultrasound examination at 11-14 weeks of gestation. Twenty-one, non-smoking normotensive nulliparous women, mean age 31.0±5.3 years and with variable levels of glycaemia at 28 weeks (120 min glucose levels 4.4-9.4 mmol/l, post 75g oral glucose) were recruited and were studied at 35.4±0.8 weeks of gestation. One woman was of South Asian descent, the others were Caucasian. One woman had ulcerative colitis treated with Mesalazine and one woman had hypothyroidism treated with thyroxin; both conditions were well controlled at the time of the study.

Study design

Assessment of the pregnant woman:

The women were admitted to the Welcome Trust Clinical Research Facility (WTCRF), Addenbrooke's Hospital, Cambridge, UK at median 35 weeks + 3 days (34^{+1} – 36^{+6}) of gestation. They arrived in the evening for an approximately 24-hour stay. During this time measurements of maternal energy substrate production and resting energy expenditure and body composition were performed as well as abdominal ultrasound to estimate the size of the fetus. There were no restrictions on food intake or exercise before the assessment, but the mothers were fasted overnight and throughout the assessments, they were only allowed to drink water.

Energy substrate production

Glucose and glycerol turnover were measured by stable isotope dilution technique. The tracers used were [U-^{13}C]-glucose (isotopic purity 98 atom %) and [1,1,2,3,3-$^{2}H_5$]-glycerol (isotopic purity 98 atom %), purchased from Cambridge Isotope Laboratories, Woburn, MA, USA. The [U-^{13}C]-glucose and [1,1,2,3,3-$^{2}H_5$]-glycerol were dissolved in 0.9% saline solution at concentrations of 24.7 and 12.4 mmol l^{-1} (4.5 and 1.2 mg $\cdot$ ml^{-1}), respectively. The intravenous solutions were sterile in microbiological cultures and pyrogen-free.

The rate of glycerol production reflects adipose tissue lipolysis. Two intravenous cannulas were inserted, one in each antecubital fossa, one for sampling and the other for infusion of stable isotope labelled compounds. A bolus of 2.25 mg kg^{-1} [$^{13}C_6$]-glucose and 0.72 mg kg^{-1} [$^{2}H_5$]-glycerol was followed by a 5-hour intravenous infusion of 0.0375 mg kg^{-1} min^{-1} and 0.012 mg kg^{-1} min^{-1} of labelled glucose and glycerol, respectively. Blood samples were obtained before the start of the tracer infusion and every 10 minutes during the last hour of the study period (total volume of 120 ml). The cannula was flushed with normal saline between samples. Samples were centrifuged immediately and plasma frozen awaiting further analysis. Production rates of glucose and glycerol were calculated from isotopic enrichments of [$^{13}C_6$]-glucose and [1,1,2,3,3-$^{2}H_5$]-glycerol obtained during periods of approximate steady state.[11] To obtain rates of appearance, the amounts of stable isotope labelled glucose and glycerol infused have to be added to the rates of production given below. No enteral contributions of glucose and glycerol were expected, since the fasting period before the examination was 12 hours. Total glucose and glycerol production was calculated by multiplying relative productions rates with maternal body weight at assessment.

Resting energy expenditure

Resting energy expenditure was estimated from respiratory gas exchange measurements made by the GEM (Gas Exchange Measurement) ventilated canopy indirect calorimetry system (GEM Nutrition, Daresbury, UK) with the woman resting and awake. The measurement was performed with the subject breathing 20-30 min through the instrument with a period of calibration with room air before and after the measurement. Any deviation from zero in the room air measurements was used to refine the subject measurements, by on average -1.45% of the unadjusted resting metabolic rate (RMR).

Maternal weight and body composition

Maternal weight was measured with electronic scales and height with a stadiometer.

BMI measured at booking was used as pre-pregnancy BMI.

Maternal body composition at 35 weeks was estimated using a three compartment model combining measurements of body mass, total body water mass and body volume in an expression derived by Siri.[12] During pregnancy there is a lower density of the fat free mass due to increased hydration[13] and the use of a 2-compartment model would overestimate the fat mass. The three compartment model assumes that the body consists of fat, water and fat-free dry mass (protein plus mineral).

Total body water measurement (TBW) was carried out using stable isotope dilution of $H_2{}^{18}O$.[14] The tracer used for TBW measurement was $H_2{}^{18}O$ water with a concentration of 10% supplied by CortecNet, Paris, France. After baseline measurements of $H_2{}^{18}O$ in saliva, a drink of $H_2{}^{18}O$ was given and further saliva samples were taken after 3, 5 and 6 hours. The concentration of Oxygen-18 in each salivary sample was determined using isotope ratio mass spectrometry (IRMS). Briefly, 0.4 ml samples were placed in 10 ml septum sealed vacutainers (Labco Ltd, High Wycombe, UK) and flush filled with 5% CO2 in nitrogen offline, using a

Gilson autosampler and a side grooved needle. Bottles were then spun overnight on blood tube rotators at room temperature to equilibrate the sample fully with the CO_2, before analysis on an AP2003 continuous flow IRMS (Analytical Precision Ltd, Northwich, UK). All measurements were made relative to SMOW (Standard Mean Ocean Water) using laboratory standards traceable to the international standard. The standard deviation of random laboratory standards, which were equilibrated in the same way and analysed with each batch of samples, was 0.15‰ (0.3 ppm) at 43.19‰ (2091.8ppm) and 0.14‰ (0.29 ppm) at 145.03‰ (2296.01ppm).[15] $H_2{}^{18}O$ dilution space was determined using the equation of Pullicino et al.[15] Total body water was calculated by reducing $H_2{}^{18}O$ dilution space values by 1% to account for the exchange of Oxygen-18 with non-aqueous Oxygen.[16]

Body volume was measured by an air displacement plethysmograph (BODPOD® Cosmed USA Inc, Concord, CA, USA).[17] The measurement of body volume was assumed not to be affected by pregnancy. After calibration the pregnant woman entered the chamber wearing a swimming suite and swim cap, and two body volume assessments were made. If the difference between the two measurements was >150 ml, a third measurement was performed and the mean of the two measurements with the best agreement were used in the calculation. Thoracic gas volume was estimated by the BODPOD® software. The software corrects the measured volume for thoracic volume and for surface area effects, yielding % fat and body mass data, computed by a Siri two-compartment expression.[18] This expression was back-calculated to yield net body volume for the three compartment model calculation.

Estimated fetal weight, and infant birth weight

Fetal weight was estimated by ultrasound examination at 34 to 36 weeks of gestation and calculated from measurements of bi- parietal diameter, head circumference, femoral length and abdominal circumference using the four parameter Hadlock model.[19] The infant weight,

was measured at delivery. Sex and gestational age independent standard deviation scores (SDS) for weight was calculated by comparison with the 1990 British growth reference. [20, 21]

Biochemical analysis

Blood glucose concentrations were measured within 30 seconds after sampling using 25 µl whole blood samples on a Y.S.I. model 2300 STAT Plus analyzer (YSI (UK) Ltd, Fleet, UK). Plasma insulin concentrations were measured using a DAKO ELISA (DAKO Ltd., Ely, UK) according to the manufacturer's instructions.

Statistics

The results are presented as median and ranges as well as mean ± SD. Variables not normally distributed were transformed into common logarithms. Relationships between maternal glucose tolerance, rates of glucose and glycerol production, insulin levels, as well as measures of maternal body composition, estimated fetal weight and infant anthropometrics were performed using Pearson bivariate correlation tests. In multiple stepwise regression analysis independent effects of variables important for estimated weight of the fetus, infant size and body composition at birth were tested. The variables in the regression analysis were chosen if significance was less than 0.05 in bivariate analysis. Analyses were performed with the IBM SPSS program version 23 (IBM, Armonk, NY, USA). Correlations and differences were considered significant at p-values <0.05.

Results

Maternal, fetal and infant characteristics

The women were examined at 35 weeks ± 6 days and until assessment the median gestational weight gain was 13 (8.3 – 31) kg. Estimated fetal weight at assessment averaged 2667 ± 300g.

The infants were born at 40.3 ± 1.0 weeks and their birth weights averaged 3502 ± 493g. Maternal rates of total hepatic glucose and glycerol production were 955 ± 180 and 235 ± 98 μmol/min, respectively. The maternal, fetal and infant characteristics and relationships between maternal weight gain, fat mass at 35 weeks and metabolic outcomes are summarized in table 1. At 35 weeks of gestation, total glucose and glycerol production rates were closely related to maternal weight, maternal fat mass, fat free mass and resting energy expenditure (table 2). Glucose and glycerol production rates were highly correlated ($r = 0.6$, $p = 0.003$) and both were related to maternal weight and fat mass at assessment. Total glucose production rate was closely related to fasting insulin ($r= 0.64$, $p= 0.001$), (table 2). Despite the wide range of maternal glycaemia as assessed by the oral glucose tolerance test at 28 weeks these measures were not related to total glucose production or total glycerol production rates.

Relationships between maternal metabolic variables and estimated fetal weight at 35 weeks gestation and birthweight

Total glucose and glycerol production rates were closely correlated with estimated fetal weight, ($r=0.71$, $p<0.001$ and 0.72, $p<0.001$, respectively) (Figures 1a, 1b and table 2). In multivariate analyses using stepwise regression model 1 glycerol production explained 52% of the variance ($r=0.72$, $p<0.001$). In model 2 total glycerol and glucose production rates were strong independent predictors of estimated fetal weight ($r=0.47$, $p= 0.018$ and $r= 0.42$, $p=0.03$, respectively) explaining 63% of the variance (table 3). Estimated fetal weight was closely related to maternal fat mass, resting energy expenditure and fasting insulin. In contrast measures of glucose tolerance obtained earlier at 28 weeks did not correlate with fetal size at 35 weeks (table 2). Infant birth weight SDS correlated with maternal rate of glucose production ($r=0.69$, $p=0.001$) (table 2) whereas the correlation with the rate of glycerol production did not reach significance ($r=0.40$, $p=0.07$).

Discussion

In this cohort of pregnant women with a normal range of glucose tolerance, rates of maternal lipolysis and glucose production correlated with estimated fetal weight at 35 weeks of gestation. The combined effects of maternal energy substrate production explained more than 60 % of the variance in estimated fetal weight which persisted after controlling for fasting insulin, resting energy expenditure, maternal size and body composition.

In late pregnancy women increase their rate of hepatic glucose production by 16-30% for the demands of the growing fetus.[11, 22] The rate of maternal glucose production in this study was strongly related to estimated fetal weight at 35 weeks of gestation. The production rate was comparable to that found in earlier studies of pregnant women in late gestation.[11, 22]

A novel finding of this study is the relationship between maternal lipolysis and estimated fetal weight. In pregnancy, although placental glucose delivery is a major factor driving fetal growth as a result of increased fetal insulin secretion and hepatic IGF-I production, glucose is not the only driver for fetal growth.[5] Previous studies have shown that increasing maternal insulin resistance in the third trimester increases the rate of lipolysis by 50%[11] and leads to an elevation of circulating triacylglycerols, which correlates with increased fetal growth.[23-25] Although the placental transport of fatty acids and triacylglycerols is limited, the loss of maternal adipose tissue in late gestation has also been shown to be associated with the exponential fetal fat accretion and weight gain.[6] The results of this study are in keeping with these findings confirming a relationship between rates of lipolysis and fetal growth.

There were no relationships between 28 weeks fasting glucose or 120 min OGTT glucose level and glucose production or estimated fetal weight at 35 weeks. The utility of OGTT data at 28 weeks to evaluate glycaemic exposures during pregnancy has been questioned in studies of continuous glucose monitoring[26, 27] and by the results of the HAPO study.[1]

In diabetic pregnancy there is data indicating enhanced gene expression related to feto-placental lipid metabolism, possibly increasing transplacental lipid delivery.[28] Placental hydrolysis of triglycerides could result in increased fetal free fatty acid levels leading to re-esterification in fetal adipocytes.[29] In a recent study, Cade et al demonstrated an increased rate of appearance of palmitate in obese women, particularly those with type 2 diabetes mellitus with and without of lipolysis index.[30]

In this study rates of glucose production and lipolysis were highly correlated and both related to maternal weight at assessment and resting energy expenditure. However, only glucose production was clearly related to fasting insulin. This suggests that the predisposition to insulin resistance predicts glucose substrate exposure to the fetus, whereas free fatty acid exposure may be more directly related to maternal fat mass.[31] Our results are also in keeping with previous studies that have shown a relationship between resting energy expenditure and maternal weight gain and estimated fetal weight.[4] However, the relationship between GPR and lipolysis remained after adjusting for resting energy expenditure.

There is a growing concern about increased maternal BMI and excess weight gain during pregnancy and the potential effect on the fetus and long term implications for the offspring.[2] Whereas initial reports from the HAPO cohort highlighted the importance of maternal glucose exposures on offspring risk for increased adiposity, macrosomia and subsequent

complications,[32] further analysis of the HAPO data have highlighted the importance of maternal pre-pregnancy BMI and maternal weight gain independent of glycaemic exposures.[2, 33] Increasing maternal weight and associated problems such as insulin resistance may expose the fetus to excess substrates others than glucose.[29] Our data supports these concerns demonstrating a positive correlation between rates of lipolysis as well as glucose production with maternal weight as well as estimated fetal weight suggesting increased glucose and free fatty acid substrate exposure to the fetus. The finding of noticeably stronger correlations between maternal energy substrate production rates and the estimated fetal weight, than between maternal weight, components of maternal body composition and size of the fetus, indicates that there is an actual relationship between maternal glucose and glycerol production and estimated fetal weight.

None of the women had gestational diabetes but three of twenty-one had impaired glucose tolerance, considered as part of a wide physiological range. Maternal insulin levels correlated to the rate of maternal glucose production, but there was no correlation between insulin and the rate of lipolysis. This is in contrast to earlier data showing an inverse relationship between the rates of glucose production and lipolysis with insulin.[11] In this earlier study there were fewer participants and the women were more lean and had less weight gain compared to the women of this study, perhaps a reason why insulin still had a regulatory role in that group of women.

Maternal weight, fasting glucose and glucose production rates at the time of assessment were important determinants of estimated fetal weight and birth weight SDS. The rate of lipolysis demonstrated a strong correlation with estimated fetal weight, but did not fully reach statistical significance in relation to infant birth weight SDS. The estimated fetal weight determined by ultrasound at the time of assessment of energy substrate production may be more closely related, than infant birth weight SDS at delivery several weeks later.

This is a complex study both in terms of the population under investigation and the methodologies used which is therefore restricted to only a small number of participants.
A strength of the study is that ultrasound assessment of estimated fetal weight was performed at the time of the extensive physiological measurements. However, due to the limited size of the study population, conclusions cannot be drawn regarding individual components of the measures (biparietal diameter, femoral length and abdominal circumference) used in the estimation of fetal weight.[34] The lack of significant relationship between maternal rate of lipolysis and the size of the newborn infant could be attributed to the small number of mother infant pairs in the context of variable placental function towards term, which would require further study.

The observation that the rate of lipolysis, as assessed at 35 weeks, is highly correlated with estimated fetal weight is important. The observations raise questions as to how increased maternal substrate availability may affect fetal growth. Rate of lipolysis, as assessed in our study, reflects availability of glycerol as substrate for gluconeogenesis in the mother but also potential availability of free fatty acids and glucose to the fetus.[29] In well controlled GDM populations maternal free fatty acid concentrations have been correlated with ultrasound estimates of neonatal abdominal circumference and neonatal fat mass at birth.[24]
This study demonstrates that within healthy pregnancies lipolysis as well as glucose production rates predict fetal weight independently of maternal size, body composition and insulin levels. This raises questions as to whether maternal substrate availability influences, not only size but fetal adiposity and subsequent disease risk in the offspring.

Figure legends

Figure 1 A. Correlation between rate of maternal hepatic glucose production and estimated fetal weight at 35 weeks of gestation.

Figure 1 B. Correlation between rate of maternal glycerol production (reflecting rate of lipolysis) and estimated fetal weight at 35 weeks of gestation.

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